

WE CLAIM:

1. A recombinant adeno-associated virus vector, which comprises:

- a) at least a portion of the adeno-associated virus genome;
- b) at least one eukaryotic based *cis*-acting regulatory sequence; and,
- c) at least one eukaryotic based nucleic acid sequence responsible for encoding a therapeutic protein or biologically active fragment thereof,

said virus vector having the property of regulating cell specific expression of said nucleic acid sequence or nucleic acid sequences upon stable transduction of a target mammalian cell.

2. A recombinant adeno-associated virus vector of claim 1 further comprising one eukaryotic based *cis*-acting regulatory sequence.

3. A recombinant adeno-associated virus vector of claim 2 further comprising one eukaryotic based nucleic acid sequence.

4. A recombinant adeno-associated virus vector of claim 1 wherein the mammalian cell is a human immune cell.

5. A recombinant adeno-associated virus vector of claim 2 wherein the mammalian cell is a human immune cell.

6. A recombinant adeno-associated virus vector of claim 3 wherein the mammalian cell is a human immune cell.

7. A recombinant adeno-associated virus vector of claim 4 wherein at least one said eukaryotic *cis*-acting regulatory sequence is chosen from the region located from about hypersensitive site I to about hypersensitive site VI, in association with the human globin gene cluster.

8. A recombinant adeno-associated virus vector of claim 7 wherein said eukaryotic *cis*-acting regulatory sequence is chosen from the region located within the group of *cis*-acting regulatory sequences consisting of hypersensitive site I, hypersensitive site II, hypersensitive site III, hypersensitive site IV, and hypersensitive site V, in association with the human globin gene cluster.

9. A recombinant adeno-associated virus vector of claim 8 wherein said nucleic acid sequence or nucleic acid sequences is responsible for encoding at least one human globin protein or a biologically active fragment thereof, chosen from the human globin gene cluster.

10. A recombinant adeno-associated virus vector of claim 5 wherein said eukaryotic *cis*-acting regulatory sequence is chosen from the region located from about hypersensitive site I to about hypersensitive site VI, in association with the human globin gene cluster.

11. A recombinant adeno-associated virus vector of claim 10 wherein said eukaryotic *cis*-acting regulatory element is chosen from the region located within the group of *cis*-acting regulatory elements consisting of hypersensitive site I, hypersensitive site II, hypersensitive site III, hypersensitive site IV, and hypersensitive site V, in association with the human globin gene cluster.

12. A recombinant adeno-associated virus vector of claim 11 wherein said nucleic acid sequence or nucleic acid sequences is responsible for encoding at least one human globin protein or a biologically active fragment thereof, chosen from the human globin gene cluster.

13. A recombinant adeno-associated virus vector of claim 6 wherein said eukaryotic *cis*-acting regulatory sequence is chosen from the region located from about hypersensitive site I to about hypersensitive site VI, in association with the human globin gene cluster.

14. A recombinant adeno-associated virus vector of claim 13 wherein said eukaryotic *cis*-acting regulatory element is chosen from the region located within the group of *cis*-acting regulatory elements consisting of hypersensitive site I, hypersensitive site II, hypersensitive site III, hypersensitive site IV, and hypersensitive site V, in association with the human globin gene cluster.

15. A recombinant adeno-associated virus vector of claim 14 wherein said nucleic acid sequence is responsible for encoding a human globin protein or a biologically active fragment thereof, chosen from the human globin gene cluster.

16. A recombinant adeno-associated virus vector of claim 15 wherein said *cis*-acting regulatory sequence comprises hypersensitive site II, associated with the human globin gene cluster.

17. A recombinant adeno-associated virus vector of claim 16 wherein said nucleic acid sequence responsible for encoding a human globin protein encodes  $\gamma$  globin or a biologically active fragment thereof.

18. The recombinant adeno-associated virus vector of claim 17 which is rAAV/HS2/ $\gamma$  globin/neo.

19. A recombinant adeno-associated virus vector of claim 18 wherein said target immune cell is chosen from the group consisting of a human hematopoietic stem cell, a human myeloid progenitor cell and a human erythroid progenitor cell.

20. A recombinant adeno-associated virus vector of claim 9 wherein said target immune cell is K562.

Sub 76 21. A recombinant adeno-associated virus vector of claim 22 wherein said target immune cell is chosen from the group consisting of a human hematopoietic stem cell, a human myeloid progenitor cell and a human erythroid progenitor cell.

22. A recombinant adeno-associated virus vector of claim 12 wherein said target immune cell is K562.

23. A recombinant adeno-associated virus vector of claim 15 wherein said target immune cell is chosen from the group consisting of a human hematopoietic stem cell, a human myeloid progenitor cell and a human erythroid progenitor cell.

24. A recombinant adeno-associated virus vector of claim 15 wherein said target immune cell is K562.

25. A recombinant adeno-associated virus vector of claim 17 wherein said target immune cell is chosen from the group consisting of a human hematopoietic stem cell, a human myeloid progenitor cell and a human erythroid progenitor cell.

26. A recombinant adeno-associated virus vector of claim 17 wherein said target immune cell is K562.

27. A recombinant adeno-associated virus vector, which comprises:

a) at least a portion of the adeno-associated virus genome;  
b) a eukaryotic based *cis*-acting regulatory sequence; and,  
c) a eukaryotic based nucleic acid sequence responsible for encoding a therapeutic protein or biologically active fragment thereof,  
said virus vector having the property of regulating cell specific expression of said nucleic acid sequence or nucleic acid sequences upon stable transduction of a human hematopoietic cell.

28. A recombinant adeno-associated virus vector of claim 27 wherein said eukaryotic *cis*-acting regulatory sequence is chosen from the region located from about hypersensitive site I to about hypersensitive site VI, in association with the human globin gene cluster.

29. A recombinant adeno-associated virus vector of claim 28 wherein said eukaryotic *cis*-acting regulatory element is chosen from the region located within the group of *cis*-acting regulatory elements consisting of hypersensitive site I, hypersensitive site II, hypersensitive site III, hypersensitive site IV, and hypersensitive site V, in association with the human globin gene cluster.

30. A recombinant adeno-associated virus vector of claim 29 wherein said *cis*-acting regulatory sequence comprises hypersensitive site IV, hypersensitive site III and hypersensitive site II.

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31. A recombinant adeno-associated virus vector of claim 30 wherein said nucleic acid sequence is responsible for encoding a human globin protein or a biologically active fragment thereof, chosen from the human globin gene cluster.

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32. The recombinant adeno-associated virus vector of claim 32 which is pJM24/vHS432A<sup>+</sup>.

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33. A recombinant adeno-associated virus vector of claim 27 which comprises a DNA sequence encoding a wild-type Fanconi anemia C complementing protein.

34. A recombinant adeno-associated virus vector of claim 33 which comprises a cis-acting regulatory element from the Rous sarcoma virus promoter region.

10 35. The recombinant adeno-associated virus vector of claim 33 which is pAAV/FACC/Neo<sup>R</sup>.

36. A method of correcting Fanconi anemia which comprises transducing hematopoietic cells from an afflicted patient with a recombinant adeno-associated virus vector, which comprises:

- 15
- a) at least a portion of the adeno-associated virus genome;
  - b) a eukaryotic based cis-acting regulatory sequence; and,
  - c) a eukaryotic based nucleic acid sequence responsible for encoding a wild-type Fanconi anemia C complementing protein,

20 such that phenotypic correction of Fanconi anemia occurs upon stable transduction of a population of the afflicted cells.

37. The method of claim 36 wherein said population of afflicted cells taken from said patient are hematopoietic cells.

38. The method of claim 37 wherein said recombinant adeno-associated virus vector of claim 33 is pAAV/FACC/Neo<sup>R</sup>.

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39. A recombinant adeno-associated virus vector of claim 27 which comprises a DNA sequence encoding a wild-type Factor IX protein.

30 40. A method of using a recombinant adeno-associated virus vector characterized by being capable of delivering and expressing in a regulated tissue specific manner at least one mammalian gene into a genome of a mammalian host cell for a therapeutic purpose comprising introducing into said host cell said recombinant adeno-associated virus vector comprising said mammalian gene, cis-acting regulatory and promoter elements of said gene, and an adeno-associated virus wherein said gene is regulated in a tissue specific manner into said mammalian host cell for effecting said therapeutic purpose.

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41. The method of Claim 40, including employing said method for treating said mammalian host having a hemoglobinopathy.

42. The method of Claim 41, including treating said hemoglobinopathy which is a hemoglobinopathy which includes sickle cell disease or beta-thalassemia.

5 43. The method of Claim 40, including employing said method for treating said mammalian host having a blood borne disorder.

44. The method of Claim 43, including treating said blood borne disorder which includes Gaucher disease.

10 45. The method of Claim 40, including employing said method for treating a disease which includes a genetic disease or an acquired disease, in which tissue specific and regulated expression of said delivered gene is desired.

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